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## **Mutations in ABCD4 cause a new inborn error of vitamin B(12) metabolism**

Coelho, David ; Kim, Jaeseung C ; Miousse, Isabelle R ; Fung, Stephen ; du Moulin, Marcel ; Buers, Insa ; Suormala, Terttu ; Burda, Patricie ; Frapolli, Michele ; Stucki, Martin ; Nürnberg, Peter ; Thiele, Holger ; Robenek, Horst ; Höhne, Wolfgang ; Longo, Nicola ; Pasquali, Marzia ; Mengel, Eugen ; Watkins, David ; Shoubridge, Eric A ; Majewski, Jacek ; Rosenblatt, David S ; Fowler, Brian ; Rutsch, Frank ; Baumgartner, Matthias R

**Abstract:** Inherited disorders of vitamin B(12) (cobalamin) have provided important clues to how this vitamin, which is essential for hematological and neurological function, is transported and metabolized. We describe a new disease that results in failure to release vitamin B(12) from lysosomes, which mimics the cblF defect caused by LMBRD1 mutations. Using microcell-mediated chromosome transfer and exome sequencing, we identified causal mutations in ABCD4, a gene that codes for an ABC transporter, which was previously thought to have peroxisomal localization and function. Our results show that ABCD4 colocalizes with the lysosomal proteins LAMP1 and LMBD1, the latter of which is deficient in the cblF defect. Furthermore, we show that mutations altering the putative ATPase domain of ABCD4 affect its function, suggesting that the ATPase activity of ABCD4 may be involved in intracellular processing of vitamin B(12).

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## Supplementary Information

### Mutations in *ABCD4* cause a new inborn error of vitamin B<sub>12</sub> metabolism

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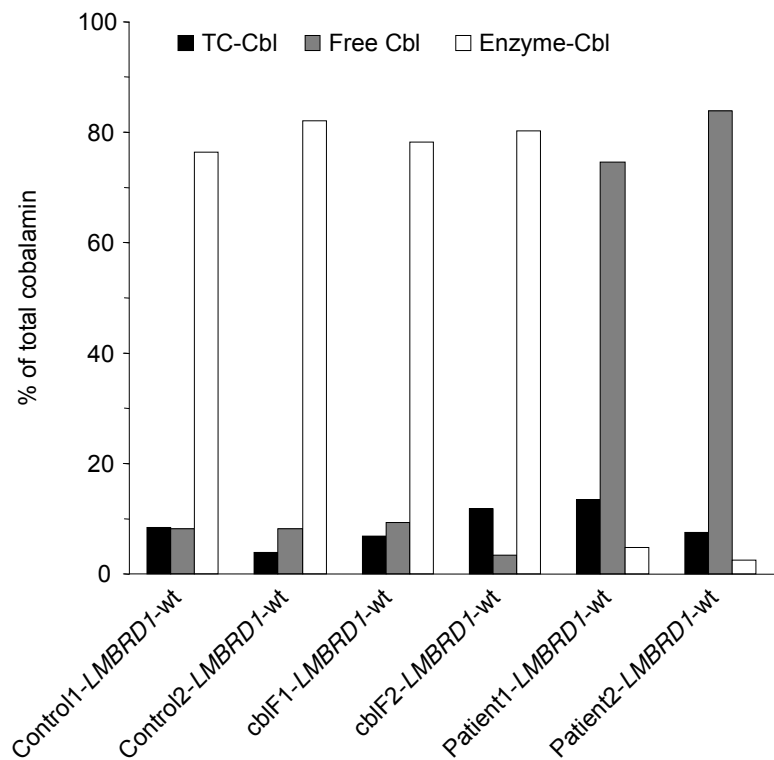
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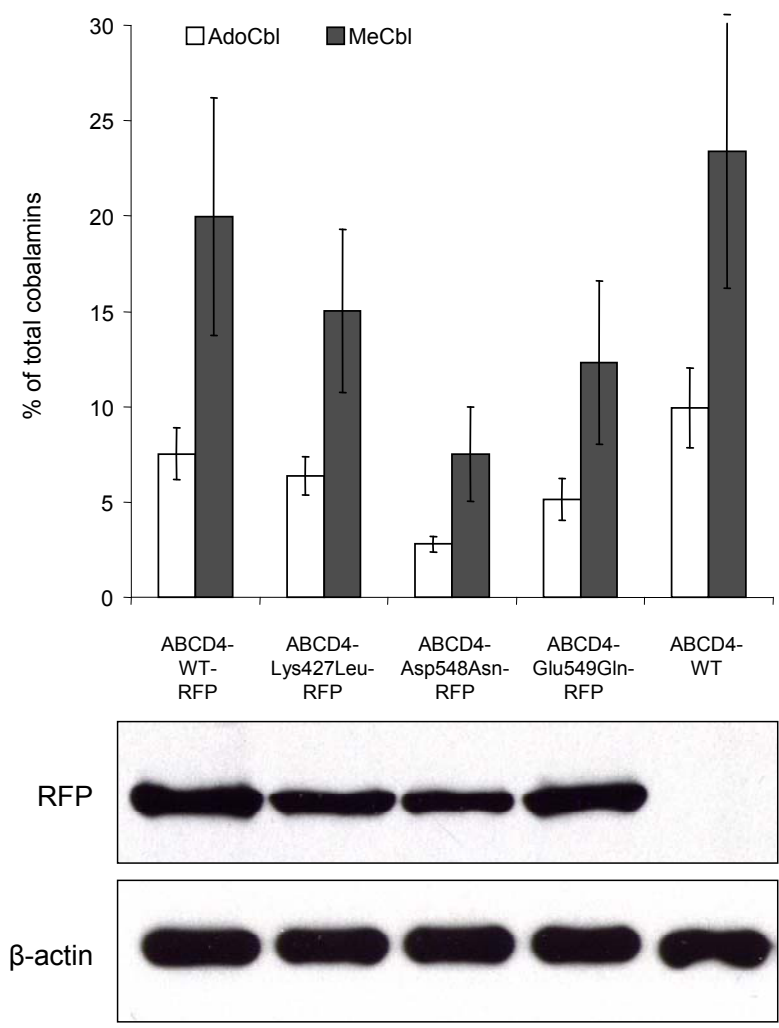
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**Supplementary Figure 1**



Distribution of free and protein-bound Cbl in *LMBRD1*-transfected control and patient cell lines. Fibroblasts from two controls, two patients with *cblF* defect, patient 1 and patient 2, which were transfected with wildtype *LMBRD1* cDNA, were incubated in a medium containing [ $^{57}\text{Co}$ ]cyanocobalamin, and cell homogenates were subjected to fast protein liquid chromatography (See Figure 1 for comparison). Transfection of control cell lines did not affect their cobalamin distributions. Transfection of both *cblF* cell lines with wildtype *LMBRD1* cDNA (*LMBRD1*-wt) corrected the cobalamin binding pattern. Transfection of both *cblJ* cell lines with wildtype *LMBRD1* cDNA did not correct the cobalamin binding pattern. All results are single determinants expressed as % of total radioactivity in all fractions in each experiment. TC-Cbl: TC-bound Cbl; Free Cbl: free form of Cbl; Enzyme-Cbl: enzyme-bound Cbl.

Supplementary Figure 2



The lower cofactors synthesis following amino-acid substitutions in the putative ATPase region does not result from a destabilizing effect or loss of expression. Immortalized fibroblasts were transfected transiently with constructs coding for *ABCD4* wild type (wt) and mutant alleles tagged with red fluorescent protein (RFP) by electroporation. Rescue of function was detected by the assay of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) synthesis. Transfection with a construct coding for untagged wild type (*ABCD4*-wt) was used as negative control. Columns represent the mean and error bars the s.d. of results from 6 (RFP-tagged) and 8 (untagged) replicate experiments with single determinations. Cell lysates were subjected to SDS-PAGE and immunoblotted with anti-RFP and anti-actin antibodies.

**Supplementary Table 1.** Biochemical findings<sup>a</sup> in cultured skin fibroblasts of both *cbfJ* patients, *cbfF* patients and unaffected controls

	Patient 1	Patient 2	<i>cbfF</i> patients	Control values
<b>Total uptake of [<sup>57</sup>Co]cyanocobalamin (pg/mg protein) and synthesis of MeCbl and AdoCbl (% distribution of total cobalamins)<sup>b</sup>:</b>				
Total uptake	214 (160 – 300)	163 (93.9 – 291)	127 (100 - 182) n=5	71 (19-142) n=32
MeCbl %	0.8 (0.2 – 1.3)	2.3 (1.5 – 2.8)	5.0 (4.0 - 5.5) n=5	60 (48-78) n=32
AdoCbl %	0 (0 – 0.1)	0.5 (0.3 – 0.8)	3.6 (3.0 - 4.3) n=5	14 (6.9-30) n=32
CNCbl %	94 (92 – 97)	87 (83 – 90)	84 (83 - 85) n=5	8.7 (3.3-18) n=32
OHCbl %	4.4 (2.8 – 5.7)	9.1 (6.6 – 12)	7.5 (7.0 - 8.0) n=5	16 (7.3-25) n=32
<b>[<sup>14</sup>C]propionate incorporation (nmol/mg protein/16h):</b>				
basal medium	0.96 (0.63 – 1.61)	1.63 (1.23-1.86)	1.11 (0.74- 1.53) n=5	11.7 (6.37 – 18.5) n=13
medium+OHCbl <sup>c</sup>	5.83 (5.00 – 7.46)	3.49 (3.06-4.19)	10.0 (8.06-15.7) n=5	12.4 (5.15- 21.4) n=13
<b>[<sup>14</sup>C]formate incorporation into methionine (nmol/mg protein/16h):</b>				
basal medium	0.08 (0.06 – 0.11)	0.45 ( 0.33 – 0.66)	0.20 (0.04-0.58) n=5	2.22 (1.11– 3.90) n=24
medium+OHCbl <sup>c</sup>	2.17 (1.84 – 2.49)	1.13 (0.75 – 1.79)	1.82 (1.37-2.40) n=5	2.58 (1.31 – 4.88) n=24
<b>[<sup>14</sup>C]methylTHF incorporation (pmol/mg protein/18h):</b>				
basal medium	40 (25 – 54)	32 (28 – 39)	43 (30 – 72) n=11	156 (79 – 221) n=5
medium+OHCbl <sup>d</sup>	60 (48 – 67)	73 (63 - 86)	297 (59 - 654) n=11	371 (300 – 546) n=5

<sup>a</sup> Values for the *cbfJ* patients are the mean and range (in brackets) of results from 3 replicate experiments, and for *cbfF* patients and controls the mean and range (in brackets) of activities from n different cell lines

<sup>b</sup> MeCbl = methylcobalamin; AdoCbl = adenosylcobalamin; OHCbl = hydroxocobalamin; CNCbl = cyanocobalamin; small amounts of further un-identified <sup>57</sup>Co-labelled compound were additionally detected (not shown)

<sup>c</sup> Parallel cell cultures were incubated for 3 days in medium supplemented with OHCbl (0.7 µmol/L), or <sup>d</sup> for 18 hours in medium supplemented with OHCbl (1.5 µmol/L) prior the assay.

**Supplementary Table 2.** Mutation analysis of *TPRG1*, *LRP2* and *ABCD4* in patient 1 and family members. Nucleotide numbering is based on the cDNA sequence with +1 corresponding to the A of the ATG translation initiation codon; WT, wild type. Patient 1 is born to parents with no history of consanguinity and has an asymptomatic sibling. Exome sequencing identified three candidate genes with compound heterozygous mutations in each. Both mutations in *TPRG1* were detected in the paternal DNA by Sanger sequencing, meaning that they were present in cis and passed down to the patient on a single allele. In fact, the c.422G>T mutation did not affect the gene product because the upstream c.183\_192del (p.Tyr61\*) mutation created a premature stop codon. Since the patient must have inherited the maternal, wild type allele, *TPRG1* mutations cannot be causative of the patient's disorder. For *LRP2*, the father and mother were heterozygous for each of the mutant alleles, but the asymptomatic sibling was found to be a compound heterozygote. Therefore, *LRP2* mutations are also not responsible for the defect. For *ABCD4*, the father and mother were again heterozygous for each of the mutant alleles, c.956A>G and c.1746\_1747insCT, respectively. However, unlike *LRP2*, the sibling did not carry either of the mutations. This pinpointed *ABCD4* as the disease-causing gene.

	<i>TPRG1</i>		<i>LRP2</i>		<i>ABCD4</i>	
<b>Father</b>	c.183_192del	c.422G>T	WT	c.10937G>A	c.956A>G	WT
<b>Mother</b>	WT	WT	c.3932G>A	WT	WT	c.1746_1747insCT
<b>Sibling</b>	WT	WT	c.3932G>A	c.10937G>A	WT	WT
<b>Patient</b>	c.183_192del	c.422G>T	c.3932G>A	c.10937G>A	c.956A>G	c.1746_1747insCT

**Supplementary Table 3.** Transfer of normal human chromosome 14 into fibroblasts of *cblJ* patient 2 rescues cobalamin cofactor synthesis. Single normal human chromosomes, tagged with a hygromycin resistance gene, were serially transferred into immortalized fibroblasts of the *cblJ* patient 2 by microcell-mediated chromosome transfer<sup>29</sup>, and the rescue of function was detected by the assay of synthesis of the cobalamin cofactors, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), from [<sup>57</sup>Co]cyanocobalamin. 30 hygromycin resistant colonies obtained after transfer of normal chromosome 14 showed rescue of AdoCbl and MeCbl synthesis whereas 36 colonies carrying twelve other transferred chromosomes (one colony with chromosome 6, 15, 22 and X, two colonies with chromosomes 2, 11 and 21, three with chromosomes 5 and 20, five with chromosome 8, six with chromosome 7 and eight with chromosome 3) showed no rescue. For comparison distribution of cobalamins is shown for immortalized *cblJ* patient 2 cells (recipient cells) and an immortalized control fibroblast cell line (both without chromosome transfer) and for 23 mouse A-9/human single chromosome hybrid cell lines (donor cell lines) each with a different single human chromosome<sup>28</sup>. Values are the mean  $\pm$  s.d. and the range in brackets of results from replicate experiments with single determinations (recipient cells and a control cell line) or of results in different cell lines (colonies, mouse human hybrid cell lines) from experiments with single determinations.

Cell line	Cobalamin distribution, % of total cobalamins*			
	MeCbl	AdoCbl	CNCbl	OHCbl
<b>Colonies obtained after transferring normal human single chromosomes into cells of <i>cblJ</i> patient 2:</b>				
30 <i>cblJ</i> colonies with normal human chromosome 14	49 $\pm$ 9 (34-64)	15 $\pm$ 5 (6-29)	13 $\pm$ 5 (5 - 24)	20 $\pm$ 6 (10-37)
36 <i>cblJ</i> colonies with 12 other normal human chromosomes	2 $\pm$ 1 (0.6-6)	0.8 $\pm$ 0.5 (0-2)	91 $\pm$ 2 (84 - 95)	5 $\pm$ 1 (2-9)
<b>Recipient, donor and control cell lines without chromosome transfer:</b>				
<i>cblJ</i> (patient 2) cells (5 experiments)	1 $\pm$ 0.4 (0.8-2)	0.7 $\pm$ 0.2 (0.4-0.9)	92 $\pm$ 1 (91-94)	5 $\pm$ 0.5 (5-6)
Control cell line (7 experiments)	49 $\pm$ 11 (33-68)	17 $\pm$ 4 (10-22)	13 $\pm$ 5 (6-22)	18 $\pm$ 5 (12-28)
23 different mouse human monochromosomal hybrid cell lines	30 $\pm$ 10 (9-46)	24 $\pm$ 6 (14-36)	17 $\pm$ 9 (6-46)	29 $\pm$ 4 (22-38)

\*MeCbl = methylcobalamin, AdoCbl = adenosylcobalamin, CNCbl = cyanocobalamin, OHCbl = hydroxocobalamin; small amounts of further un-identified <sup>57</sup>Co-labelled compound were additionally detected (not shown)

**Supplementary Table 4.** Filtering strategy for the called variants in patient 2. Using MAQ and SAMtools, in total 417198 variations were found. After filtering for a minimal phred-like consensus quality score >15 and minimal coverage of 4 reads, 164578 variations remained. A stringent filter step discarding known variations using dbSNP and 1000 genomes data reduced the list to 25418 variations of which 729 covered chromosome 14. Further filtering for allele frequency > 25%, protein changing variations or variations +/- 10 nts close to the 3'/5' splice sites reduced the list to 9 candidate variations.

Filtering step	Count
Total count of variations	417198
Minimal quality (>4 reads; Qual>15)	164578
Not in dbSNP and 1000genomes	25418
On chromosome 14	729
Protein Change or within 10 bp 3'SS/5'SS + Allelfreq > 25%	9



**Supplementary Table 5.** Candidate gene list for patient 2 based on whole exome sequencing and stringent filtering. In total 9 candidate variations were detected. Four of them were found to be rare variants by searching the Exome Variant Server (NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: <http://snp.gs.washington.edu/EVS/>) [Sept 2011]). The *FOXN3* variant was predicted to be benign by the Polyphen software. The splice site variant in *GALC* is not very likely to be pathogenic because it does not affect the conserved splice positions and also is embedded in an poly-A stretch leading to an A<sub>(11)</sub> to A<sub>(10)</sub> shortening. The remaining variations are a 2-bp deletion in *TRIP11* resulting in a premature termination codon and two different mutations in the gene *ABCD4*: Gly486Cys which was predicted as possibly damaging (Polyphen) and a splice site mutation affecting the highly conserved position +1. This pinpointed *ABCD4* as the disease-causing gene.

Chr.	g.DNA_change	Qual.	Depth	Allele	NHLBI Exome		Gene	CCDS	c.DNA_change	Prot_Change	Polyphen
				Freq.	Variant	Server (MAF)					
14	74756186C>A	255	120	38			<i>ABCD4</i>	CCDS9828.1	c.1456G>T	Gly486Cys	possibly damaging
14	74763035C>A	177	39	46			<i>ABCD4</i>	CCDS9828.1	c.542+1G>T		
14	96752219G>A	255	131	41	0,0007		<i>ATG2B</i>	CCDS9944.2	c.6110C>T	Pro2037Leu	probably damaging
14	24113634A>G	255	253	44	0,007		<i>DHRS2</i>	CCDS41927.1	c.557A>G	Asn186Ser	
14	89647123G>A	255	84	46			<i>FOXN3</i>	CCDS41977.1	c.839C>T	Ala280Val	benign
14	88417096delA	678	148	76			<i>GALC</i>	CCDS9878.2	c.1162-4delT		
14	105609325C>T	98	27	63	0,003		<i>JAG2</i>	CCDS9998.1	c.3424G>A	Gly1142Arg	possibly damaging
14	64625344T>C	255	200	43	0,02		<i>SYNE2</i>	CCDS9761.2	c.15794T>C	Val5265Ala	possibly damaging
14	92471348AGdel	11770	389	40			<i>TRIP11</i>	CCDS9899.1	c.2972_2973delCT	Ser991*	

**Supplementary Table 6.** Statistical significance (p -values) of rescue of function presented in Fig 3. Differences in adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) synthesis were evaluated after transient transfection of immortalized fibroblasts with different wild type (wt) and *ABCD4* mutant constructs using transfection with an empty pTracer vector (vector only) as negative control.

The statistical significance was tested by using the unpaired t-test (two tailed), with Welch's correction for unequal variances, and GraphPad Prism software (version 4). P-values less than 0.05 were considered to indicate statistical significance

Cell line transfected	Comparison between		p-values for synthesis of	
	construct 1	& construct 2	AdoCbl	MeCbl
Patient 1	vector only	<i>ABCD4</i> -wt	p = 0.005	0.0002
Patient 2	vector only	<i>ABCD4</i> -wt	p = 0.0009	0.010
Patient 1	<i>ABCD4</i> -wt	<i>ABCD4</i> -c.956A>G	p = 0.016	0.0003
Patient 2	<i>ABCD4</i> -wt	<i>ABCD4</i> -c.956A>G	p = 0.001	0.029
Patient 1	<i>ABCD4</i> -wt	<i>ABCD4</i> -c.1456G>T	p = 0.005	0.0002
Patient 2	<i>ABCD4</i> -wt	<i>ABCD4</i> -c.1456G>T	p = 0.0007	0.013
Patient 1	vector only	<i>ABCD4</i> -c.1456G>T	p = 1.00	0.79
Patient 2	vector only	<i>ABCD4</i> -c.1456G>T	p = 0.42	0.007*
Patient 1	vector only	<i>LMBRD1</i> -wt	p = 0.57	0.79
Patient 2	vector only	<i>LMBRD1</i> -wt	p = 0.38	0.21
<i>cbID</i>	vector only	<i>ABCD4</i> -wt	p = 0.42	0.98
<i>cbIF</i>	vector only	<i>ABCD4</i> -wt	p = 0.005	<0.0001

\* significant but very low increase from 5-9% to 11-16% of the mean *ABCD4*-wt values.

**Supplementary Table 7.** Functional peroxisomes are not required for intracellular synthesis of the cobalamin cofactors adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl). Three fibroblast cell lines with severe deficiency of peroxisomal biogenesis and deficient import of catalase due to homozygosity for a mutation in different *PEX* genes and one control cell line (experimental control) were incubated for 4 days in a medium containing 10% human serum and 25 pg/ml [<sup>57</sup>Co]cyanocobalamin. Then total uptake of [<sup>57</sup>Co]cyanocobalamin and cobalamin coenzyme synthesis were estimated as described under Methods.

Cell line genotype (homozygous)	Total uptake <sup>§</sup> pg/mg protein	Distribution of cobalamins, % of total cobalamins <sup>§</sup>				
		OHCbl	CNCbl	AdoCbl	MeCbl	unknown
Cell line 1: mutation in <i>PEX1</i> c.2097_2098insTC>T / p.872*	63.1	12.1, 10.9	5.1, 6.4	<b>11.4, 11.7</b>	<b>69.7, 69.6</b>	1.7, 1.5
Cell line 2: mutation in <i>PEX2</i> c.115C>T / p.Gln39*	90.2	10.2, 11.4	7.3, 7.1	<b>13.3, 12.8</b>	<b>67.2, 66.4</b>	2.0, 2.3
Cell line 3: mutation in <i>PEX6</i> c.685_686insAG / p.Ser232Hisfs*15	85.0	13.4, 12.7	5.8, 4.9	<b>14.2, 13.6</b>	<b>64.7, 66.7</b>	1.9, 2.1
Experimental control	72.1	15.2, 16.8	6.8, 8.0	<b>8.8, 10.9</b>	<b>67.1, 61.6</b>	2.1, 2.7
<b>32 reference cell lines: mean</b>	70.8	15.6	8.7	<b>14.1</b>	<b>60.3</b>	1.3
	range	(18.7-142)	(7.3-24.6)	(3.3-18.3)	(6.9-29.9)	(47.7-78.5) (0.1-3.1)

<sup>§</sup>values obtained in *PEX* mutant cell lines and the experimental control are single determinations within one experiment

OHCbl =hydroxocobalamin; CNCbl =cyanocobalamin; AdoCbl = adenosylcobalamin; MeCbl =methylcobalamin

## Supplementary Note

### Case reports

**Patient 1** was born at 40 weeks gestation by emergency C-section for fetal distress. The birth weight was 2.565 kg (1<sup>st</sup> centile), length was 47 cm (1<sup>st</sup> centile) with weight for height at the 23<sup>rd</sup> centile, head circumference was 31.8 cm (<1<sup>st</sup> percentile). She had respiratory distress and required supplemental oxygenation by oxyhood. APGAR scores were 6 at 1 minute and 8 at 5 minutes. She was placed on antibiotics to treat a possible pneumonia because of an infiltrate seen on chest X-rays. She was noted to have hypotonia, lethargy, poor feeding, periodic breathing, and episodes of posturing. There was evidence of bone marrow suppression requiring red blood cells and platelet transfusions. Newborn screening showed an elevated C3 (propionyl) carnitine at 11.9 (reference <5)  $\mu\text{mol/L}$  (the newborn screening program did not have a cut-off for low methionine). Initial laboratory testing failed to show metabolic or lactic acidosis or ketonuria. The infant was started on hydroxocobalamin and transferred to a tertiary facility at 2 weeks of age. On admission, plasma amino acids indicated low methionine of 5  $\mu\text{mol/L}$  (reference 17-53), absent free homocysteine, elevated total plasma homocysteine at 20  $\mu\text{mol/L}$  (reference <12). The serum methylmalonic acid was 20.25  $\mu\text{mol/L}$  (reference <0.4). Urine organic acids indicated an isolated increase in methylmalonic acid at 154 mmol/mol creatinine (reference <5). Blood counts were significant for neutropenia (absolute neutrophil count 0.3 K/ $\mu\text{L}$ , reference 1.5-10 K/ $\mu\text{L}$ ), thrombocytopenia (55-106 K/ $\mu\text{L}$ , reference 150-400 K/ $\mu\text{L}$ ), while the previous anemia had resolved with transfusions. The patient was started on Colony Stimulating Factor that corrected neutrophil and platelet counts. The child was continued on a regular diet (breast milk), hydroxocobalamin (2 mg per day) IM injections, methylcobalamin (2 mg p.o. twice a day), methylTHF (3 mg twice a day), pyridoxal phosphate (35 mg twice a day), betaine (100 mg/kg per day divided into 3 doses). With this treatment, the plasma amino acids normalized completely, as did total plasma homocysteine and serum methylmalonic acid levels. Additional problems identified were a heart murmur (caused by 2 small atrial level shunts that were not significant hemodynamically, and were no longer present at 1 year of age) and gastroesophageal reflux. Brain imaging and spectroscopy were unremarkable. Clinically, she stopped requiring oxygen, started eating orally and was discharged home at 36 days of age.

Following discharge from the hospital, the patient did well with no other hospitalizations, except one at 7 months of age for the elective repair of a left inguinal hernia. From a developmental standpoint, the child started walking unassisted at 12 months, spoke her first word at 15 months and, at two years of age speaks about 30 words without sentences. She is being followed by a speech therapist. At 2 years of age, she has an unrestricted diet and growth parameters are at the 5<sup>th</sup> percentile. The therapy consists of hydroxocobalamin (2.5 mg IM twice per week), methylcobalamin (2 mg p.o. twice a day), methylTHF (3 mg twice a day), pyridoxal phosphate (35 mg twice a day). Betaine was stopped at 6 months of age. With this therapy, plasma amino acids and total plasma homocysteine are persistently within the reference range, while methylmalonic acid is mildly elevated in serum (0.68-1.45  $\mu\text{mol/L}$ , reference <0.4  $\mu\text{mol/L}$ ) and urine (6-13 mmol/mol creatinine, reference <5 mmol/mol creatinine).

**Patient 2** was born as the second child of non-consanguineous German parents at 40 weeks of gestation by C-section because of a discrepancy between the maternal pelvis and the fetal head circumference. Birth weight was 3040 g (12<sup>th</sup> centile), length 48 cm (10<sup>th</sup> centile), head circumference 35.5 cm (50<sup>th</sup> centile), and the APGAR scores were 9 and 10 at 5 and 10 minutes, respectively. On the second day of life he had raised body temperature and tachypnea, and sepsis therapy was started. He was referred to a tertiary care centre, where

physical examination revealed hypertelorism, micrognathia, wide inter-mamillary distance, a bell-shaped thorax, horizontal ribs and short extremities. Cardiac catheterization demonstrated an atrial septal defect, coarctation of the aorta, a small left ventricle, an enlarged right ventricle and pulmonary hypertension. He was treated with antibiotics, diuretics and digoxin. Because of feeding difficulties he required a nasogastric tube.

Newborn screening was reported to be unremarkable. Laboratory testing revealed normocytic normochromic anemia with a haemoglobin level of 12 g/dl, mean corpuscular volume 101fl, and mean corpuscular hemoglobin of 35.2 pg. He received two red blood cell transfusions. Chromosome analysis revealed a normal male karyotype.

The clinical course during the first months of life was complicated by feeding difficulties, generalized hypotonia and developmental delay. Cerebral ultrasound revealed mild cerebral atrophy at 4 months of age. Repeated cardiac catheterization at 6 months of age revealed increased narrowing of the aortic isthmus and a hemodynamically relevant type II atrial septal defect. Therefore, corrective surgery of the aorta and closure of a patent ductus arteriosus was performed. At the age of 13 months, Noonan syndrome was suspected because of failure to thrive, facial dysmorphism, developmental delay and cardiac anomalies. At this age, his weight was 6400g (below 1<sup>st</sup> centile), length 64 cm (below 1<sup>st</sup> centile), and head circumference 43 cm (above 97<sup>th</sup> centile). Mutation analysis of the *PTPN11* gene for Noonan syndrome did not reveal a mutation. Serum amino acids were reported as normal. Skeletal X-rays of pelvis, hand, knee and spine revealed a delayed bone age, but no specific skeletal anomaly.

At the age of 16 months, methylmalonic aciduria was noted, plasma total homocysteine was 50 (reference <15)  $\mu\text{mol/l}$ , and plasma cobalamin was 815 pg/ml (reference 190-880 pg/ml). Fibroblast studies revealed findings consistent with the *cbfF* defect. At the age of 18 months, he received his first dose of hydroxocobalamin, 1 mg intramuscularly. The homocysteine levels dropped to 15  $\mu\text{mol/l}$ . Monthly injections with hydroxocobalamin were started. The patient showed some progress of his psychomotor development and started to speak two word sentences at the age of 20 months. At the age of 22 months, he had a bilateral inguinal hernia repair and a bilateral orchidopexia for cryptorchidism. At the age of 2 ½ years, he had a complicated febrile convulsion. At the age of 5 years, he was treated with hydroxocobalamin injections 1 mg every 4 to 5 weeks intramuscularly. He showed gradual progress in his psychomotor development, but was still underweight (13.0 kg, below 1<sup>st</sup> centile) and short (92 cm, below 1<sup>st</sup> centile).

At the age of 7 years, he developed pneumococcal septic arthritis of the right hip, during an episode of neutropenia (leucocyte count 3700/ $\mu\text{l}$ , absolute neutrophil count 1300/ $\mu\text{l}$ ), requiring surgery of the hip and prolonged systemic antibiotic therapy. The hospital course was complicated by a mediastinal hemorrhage (hemoglobin dropped to 7 g/dl, hematocrit 21%) and paresis of the right phrenic nerve, most likely caused by a dislocation of a central venous catheter. He therefore required a red blood cell transfusion. Intramuscular injections with hydroxocobalamin were continued at a dose of 1.0 mg every three weeks. Currently, at the age of seven and a half years, levels of both plasma homocysteine (15.5  $\mu\text{mol/l}$ ) and serum methylmalonic acid (134  $\mu\text{g/l}$ ) remain elevated.

### Comparison with the *cbfF* phenotype

The findings in the two *cbfJ* patients show similarities to these seen in the thirteen reported patients who fall into the *cbfF* complementation class<sup>1</sup>. The *cbfF* patients typically had elevations of both homocysteine and methylmalonic acid, although only elevated methylmalonic acid was described in the original patient. Most *cbfF* patients were diagnosed in the first year of age, some following positive newborn screening, although one was not diagnosed until eleven years of age. Features seen in some *cbfF* patients, and also seen in at least one of the two *cbfJ* patients, include feeding difficulties, anemia and pancytopenia, unusual facial appearances, and congenital

heart defects. Thus, it would be hard to distinguish *cb1F* and *cb1J* patients on clinical and laboratory grounds alone. A definitive diagnosis requires somatic cell complementation analysis and/or sequencing of the *LMBRD1* and *ABCD4* genes.

1. Watkins D. & Rosenblatt D.S. Inherited Disorders of Folate and Cobalamin Transport and Metabolism. In C. Scriver et al (eds): Metabolic and Molecular Bases of Inherited Disease -- OMMBID. [www.ommbid.com](http://www.ommbid.com), New York, McGraw-Hill, Chap. 17, August, 2011.